

The Results of Prolonged Action of GLP-1 on Some Metabolic Parameters

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Glucagon-like peptide (GLP-1) is widely considered as a potential drug against diabetes mellitus and obesity. It strongly stimulates the pancreas to produce and release insulin, even a few minutes after meal consumption. Because of this action, GLP-1 has been called an „incretin hormone”. Moreover, GLP-1 decreases the level of glucose in the blood, independently of insulin. An obstacle to clinical application is the very short half-time of GLP-1 degradation by dipeptidyl-peptidase IV in the blood. This research was aimed at tracing all possible changes evoked by long-term application of GLP-1 in rats and comparison of two methods of application: osmotic minipumps and daily injections. In the 13-day experiment, samples of blood, muscle and liver from 24 male Wistar rats were used. Analysis included glycogen, glucose, triglycerides, free fatty acids, cholesterol, triiodothyronin, thyroxin, insulin and glucagon concentrations. The results show a lack of significant differences between both methods of application. We suggest this may be evoked by adaptation of the organism to the prolonged action of GLP-1.

Key words: Glucagon-like peptide-1, osmotic minipumps, lipids, carbohydrates, rats, long-term action.

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Glucagon-like peptide-1 has been considered as a potential drug against diabetes mellitus and obesity because of its wide range of activity in organisms (EGAN *et al.* 1994; BLÁZQUEZ *et al.* 1998; SCHWARTZ *et al.* 2000). This peptide is believed to be the strongest stimulatory agent for pancreatic β cells (AHRÉN *et al.* 1995). Because it stimulates these cells to secrete insulin, it is called an “incretin factor”. Acting independently from insulin, GLP-1 also decreases the glucose level in blood, but only in the presence of glucose. Moreover, this peptide has been detected in the central nervous system, where it contributes to the reduction of food intake and loss in body weight, affecting local hypothalamic neurons (GUNN *et al.* 1997; VAN DIJK & THIELE 1999; OKA *et al.* 1999; NASLUND *et al.* 2004; KNAUF *et al.* 2008).

Previous studies considering the mechanism of GLP-1 action and function led to controversial and contradictory conclusions (RUIZ-GRANDE *et al.* 1992; TOFT-NIELSEN *et al.* 1999; own data, unpublished). The question of its involvement in the process of lipid regulation still remains unexplained. However, as shown by the results of an

experiment on isolated adipocytes, application of GLP-1 accelerates the lipolytic process (RUIZ-GRANDE *et al.* 1992). Moreover, in our unpublished experiment, we measured lipolysis by the amount of glycerol released into the incubation medium. GLP-1 did not affect the lipid profile under *in vivo* conditions. Therefore, both its therapeutic utility in the treatment for obesity is still indefinite and its involvement in the process of appetite reduction still remains unclear. It has been suggested that GLP-1 appearing in the circulation after food consumption does not influence the neurons responsible for hunger (VAN DIJK & THIELE 1999).

The GLP-1 receptor belongs to the metabotropic receptor family. It is associated with G proteins in the inner side of the cell membrane. The binding of GLP-1 ligands or its analogues to the receptor enhances the concentration of cAMP in the cell (THORENS 1992). The receptor has been found also in the kidneys, stomach, lungs (THORENS 1992) and hypothalamus (BLÁZQUEZ *et al.* 1999) but mainly in pancreatic β cells (TORNEHAVE *et al.* 2008).

GLP-1 as a therapeutic medium against diabetes mellitus and obesity is regarded as problematic because of its short half-time, lasting 3 to 11 minutes. After this time, the peptide is degraded by dipeptidyl-peptidase IV (DPP IV). Hence, because of its short-term effect, it cannot be used as an auxiliary medication for diabetes and anti-obesity treatment.

The product of GLP-1 degradation, (GLP-1(7-39)), is an antagonist of GLP-1's receptor (MENTLEIN *et al.* 1993; KIEFFER *et al.* 1995). Therefore, any stable analogues of GLP-1 and blockers of dipeptidyl-peptidase IV, preventing or delaying the degradation of the peptide, are still being tested (KIM *et al.* 2003; KENDALL *et al.* 2005; DEFRONZO *et al.* 2005; GAO *et al.* 2009; HOLST & SEINO 2009).

The following experiment, under *in vivo* conditions, was also planned to compare two techniques of GLP-1 application. The first method was based on daily injection, whereas the second was focused on maintaining a relatively unchanged level of GLP-1 in the organism. We used Alzet pumps because of the constant levels of compounds applied in this way, also because it decreases the stress of animals caused by handling and daily injections. Moreover, drug delivery systems ensure that constant levels of compounds are maintained at therapeutic level. We concur that the pumps are more correct for long-term metabolic experiments because of the sustenance of stable amounts of used substances, previously placed in the body of animals.

A previous study was conducted on human volunteers who received GLP-1 applied from osmotic pumps inserted subcutaneously for a prolonged period (TOFT-NIELSEN *et al.* 1999). However, the authors reported on the changes in the feeling of satiety and feeding preferences, but did not measure any metabolic parameters in the tissue samples.

The goal of this study was to examine the effects caused by long-term activity of GLP-1 applied into the peritoneal cavity of rats. Searching for hormonal, lipid and carbohydrate changes over several days seemed to be one of the most interesting issues in the experiment. The rats were exposed to the long-term action of the peptide which was applied into the peritoneal cavity. The changes were studied throughout the 13 day duration of the study.

Material and Methods

24 male *Wistar* rats from the Department of Toxicology of the University of Medical Sciences in Poznań, at approximate weight of 150 ± 5 g, were used in this study. The animals were kept under standard conditions (12:12 light-dark cycle, 25°C), with free access to a rodent chow diet and

water. Osmotic infusion minipumps Alzet (Alzet Osmotic Pumps, Durect Corporation, USA) were placed intraperitoneally in 12 of them in deep anesthesia (ketamine 80 mg/kg b.w. + xylazine 10 mg/kg b.w., i.p.). Half of the rats received GLP-1 (Sigma-Aldrich Co., USA) (0,6 nmol/24h/rat; $n = 6$), the rest an 0.9% solution of NaCl. 12 animals were exposed to a daily injection of GLP-1 (0.6 nmol/24h/rat; volume 200 μl ; $n=6$), or 200 μl of 0.9% NaCl solution intraperitoneally. The concentration of applied GLP-1 was estimated at 0.23 ± 0.02 $\mu\text{l}/\text{hour}$ according to the producer's advice. After 13 days of the experiment the rats were weighed (200 ± 15 g) and decapitated. Samples of the livers, muscles (quadriceps muscle) were collected and frozen in liquid nitrogen and stored in -20°C until analysis. Samples of blood were collected after decapitation of animals, kept in 4°C for 30 minutes, and then centrifuged. The blood clot was discarded and serum samples were collected and frozen in -20°C until further analysis.

The concentrations of insulin, glucagon, thyroxine (T3), triiodotyronine (T4) (Linco Research, USA), triglycerides (FOSTER & DUNN 1973), total cholesterol (RICHMOND 1973), free fatty acids (DUNCOMBE 1964) and glucose (HUGGET & NIXON 1957) were measured in the blood serum. The concentration of TG was additionally measured in the muscle tissue taken from the quadriceps muscle and in the liver of rats.

The results were analyzed using Students *t*-tests at $P \leq 0.05$.

Results

There were no side effects of GLP-1 on rat behavior throughout the duration of the experiment. During this time the body mass of animals tested increased (50 ± 10 g/rat) in both groups of rats.

Figure 1 shows the concentration of insulin in the serum of rats supplied every day with GLP-1 at

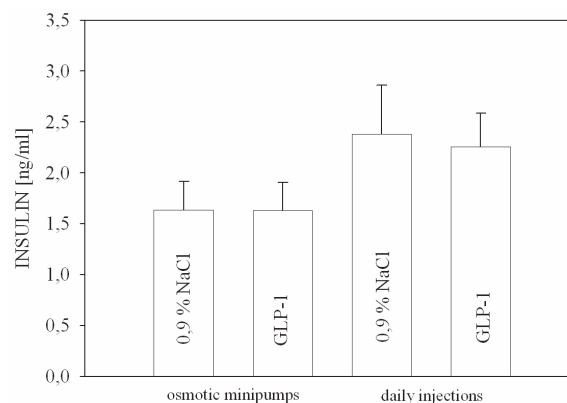


Fig. 1. The effect of prolonged action of GLP-1 on the insulin level in the blood serum of rats. The results are presented as mean values \pm SEM; ($n=6$).

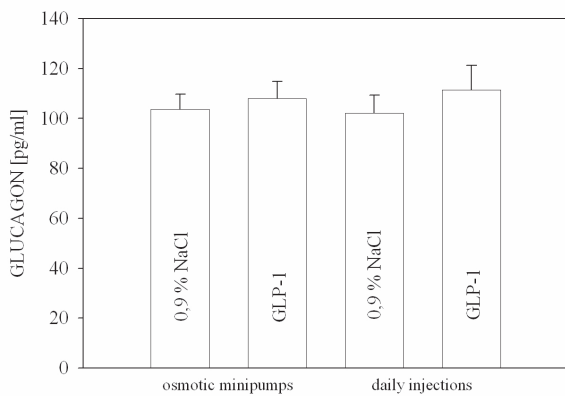


Fig. 2. The effect of prolonged action of GLP-1 on the level of glucagon in the blood serum of rats. The results are presented as mean values \pm SEM; (n=6).

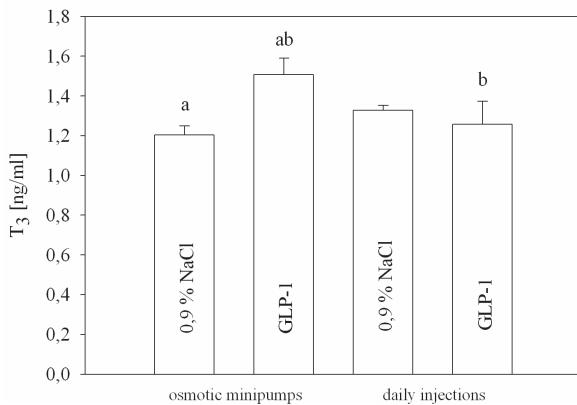


Fig. 3. The effect of prolonged action of GLP-1 on the level of triiodothyronin in the blood serum of rats. The results are presented as mean values \pm SEM; (n=6). The values assigned with the same letter are statistically different at $P \leq 0.05$.

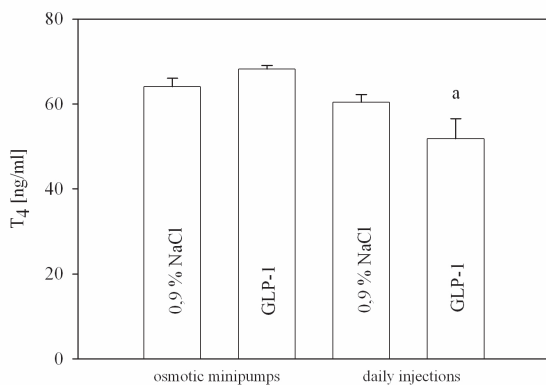


Fig. 4. The effect of prolonged action of GLP-1 on the level of thyroxin in the blood serum of rats. The results are presented as mean values \pm SEM; (n=6). The value assigned as "a" is statistically different ($P \leq 0.05$) from other bars.

a concentration of 0.6 nmol/24h/rat or 0.9% NaCl. It was found that the level of insulin in the blood serum of animals injected daily was about 40% higher compared with rats supported with

minipumps. Corresponding glucagon concentrations determined in both groups of rats achieved about 100 pg/ml and the differences between them were not statistically significant (Fig. 2).

In our study we did not find a clear effect of GLP-1 on any metabolic process. An increase of T3 and T4 was found in the group of rats supplied with minipumps, however only the increase of T3 was significant. Interestingly, this type of application, where the minipumps were used, led to a clear, statistically significant decrease in the level of both thyroid hormones (Figs 3 & 4).

The lipid concentration in the blood was analyzed since GLP-1 is considered as a potential drug against obesity (RUIZ-GRANDE *et al.* 1992; GUNN *et al.* 1997; VAN DIJK & THIELE 1999; OKA *et al.* 1999; NASLUND *et al.* 2004; KNAUF *et al.* 2008). The prolonged action of this peptide, delivered by minipumps or injected, did not affect the concentrations of cholesterol (Fig. 5), free fatty acids (Fig. 6) and triglycerides in the blood serum (Fig. 7). Only an insignificant increase of cholesterol level in the blood caused by GLP-1 (Fig. 5) was found in both groups. There were also no significant changes in

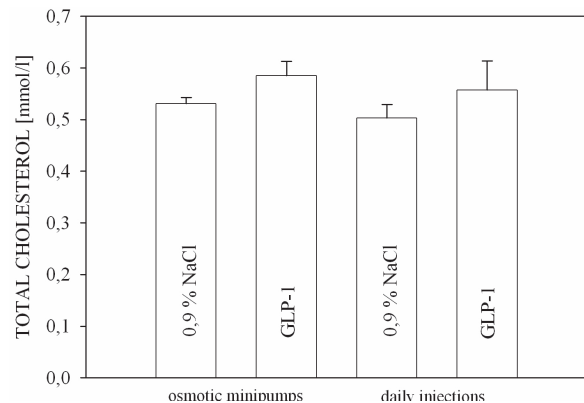


Fig. 5. The effect of prolonged action of GLP-1 on the level of total cholesterol in the blood serum of rats. The results are presented as mean values \pm SEM; (n=6).

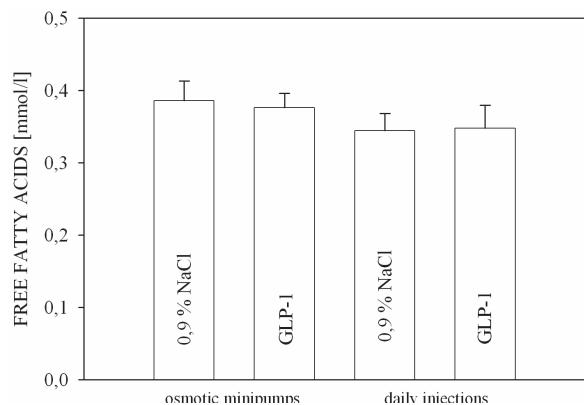


Fig. 6. The effect of prolonged action of GLP-1 on the level of free fatty acids in the blood of rats. The results are presented as mean values \pm SEM; (n=6).

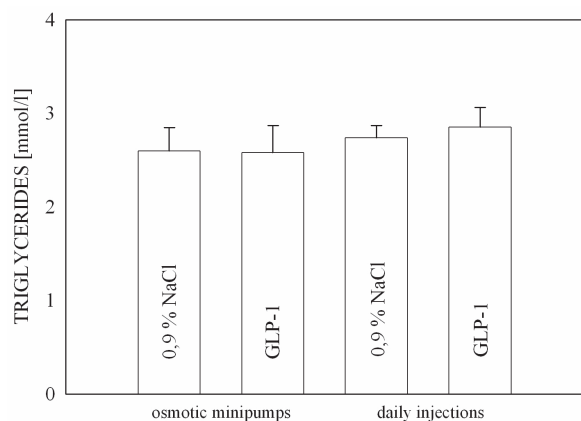


Fig. 7. The effect of prolonged action of GLP-1 on the level of triglycerides in the blood of rats. The results are presented as mean values \pm SEM; (n=6).

TG concentration in muscles and liver (not shown). The concentration of glucose in the blood and glycogen in the liver did not change as well (data not shown).

Discussion

The long-term action of GLP-1, applied into the peritoneal cavity using daily injections and osmotic pumps, was tested on rats during a 13 day study.

Insulin and glucagon concentrations in the blood of the rats suggested little effect of prolonged GLP-1 action on the pancreas. The blood serum of animals with minipumps inserted into the peritoneal cavity showed an elevated level of insulin, but these results were not statistically significant in either control or experimental groups. However the impact of GLP-1 on pancreatic activity was obvious in experiments under in situ conditions on perfused pancreata of normal (own data, unpublished) and diabetic (SHEN *et al.* 1998) rats. The long-lasting action of higher, non-physiological concentrations GLP-1 may lead to the development of adaptive mechanisms in the whole organism.

Although it has been reported that the long-term action of GLP-1 evokes numerous anabolic effects in human volunteers (AHRÉN *et al.* 1995; KNAUF *et al.* 2008) our results have not confirmed this presumption. Daily injections of GLP-1 caused the opposite reaction – a small (Fig. 3) or distinct (Fig. 4) decrease in the concentrations of T3 and T4 in the blood. The concentrations of T3 and T4 in the blood of rats with inserted minipumps indicated a quite clear or less distinct increase in these hormones. We suppose that modified levels of T3 and T4 in the blood may be the result of adaptation of

tissues to GLP-1 in the regulation of energy balance.

An analysis of lipids in the blood serum and in some tissues was conducted because published data reported disturbances in insulin and GLP-1 secretion in obese people as well enhanced levels of lipids in the blood of such patients. RANGARATH *et al.* (1999) proved that a disorder in secretion of GLP-1 in obese individuals contributed towards obesity. They also showed that high lipid levels in the blood may suppress the sensitivity of L cells towards diet factors, leading to a decrease in GLP-1 concentration, involving hunger and thirst and diminishing insulin secretion from the pancreas. In consequence the mechanisms regulating food intake and storage become disordered, contributing to increased body mass and obesity.

A study considering the effects of GLP-1 has been carried out on patients with type 2 diabetes mellitus. In all cases, after prolonged exposition to GLP-1 changes in lipids, carbohydrates, and hormonal profiles were observed (TOFT-NIELSEN *et al.* 1999). This peptide applied continuously did not affect the cardiovascular system in the manner of other anti-diabetic drugs. Moreover, GLP-1 decreased appetite and diminished glucose levels in the blood in the postprandial phase, pointing to its importance in diabetes mellitus therapy.

In our study we also observed that GLP-1 did not affect the lipid profile nor lipid concentration in blood, independent of the method of application. Only the total cholesterol concentration in the blood showed a small but statistically unimportant increase in the two variants of the experiment.

We found that the method of GLP-1 application is an important factor influencing the results of the experiment. In two cases (T3, T4) osmotic pumps releasing GLP-1 showed different results ($P \leq 0.05$), compared with daily injection of this peptide. Stable and continuous maintenance of a proper concentration of the peptide using Alzet osmotic minipumps can probably achieve a stronger biological effect compared with repeatable application. This is an important aspect of rapid GLP-1 utilization in the tissue and the antagonistic role of the degraded GLP-1 product.

The obtained data suggest that GLP-1 can be viewed as a potential drug against obesity. Interestingly, its effects increased temporarily when this peptide was injected in single doses at high concentrations (unpublished research). In contrast, the long-lasting, permanent application of GLP-1 did not show such an impact on the rats' metabolism. In this case, adaptation of the cells to an increased concentration of the peptide is probably involved.

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